The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

VITAMIN A

SUMMARY REPORT

1. Vitamin A is the generic term designating any compound with beta-ionone structure having qualitatively the biological activity of retinol. The recommended posology is 10 000 to 20 000 IU/kg bw in calves and adult cattle, 6 000 IU/kg bw in sheep and goats, 1 500 IU/kg bw in horses, 2 000 to 3 000 IU/kg bw in pigs, poultry and rabbits either by oral, intramuscular and subcutaneous routes. Vitamin A is generally used as esters such as acetate, propionate and palmitate. It is also authorized as feed additive at the maximum concentration of 13 500 IU/kg of complete feed for all domestic species and 25 000 IU/kg in milk replacer for calves.

2. Vitamin A activity is today expressed in units of weight as retinol equivalents, 1 µg retinol equivalents corresponding to 1 µg retinol. The previously used definition as International Unit (IU) corresponds to 0.3 µg retinol. In the European Pharmacopoeia, the activity of vitamin A is still expressed in International Units, one International Unit of vitamin A corresponding to the activity of 0.344 µg of all-trans retinyl acetate. The activity of the other vitamin A compounds is calculated stoicheiometrically so that 1 International Unit corresponds to the activity of 0.300 µg of all-trans retinol, 0.359 µg of all-trans retinyl propionate or 0.550 µg of all-trans retinyl palmitate.

   The observed biopotency is 3.33 million IU/g of vitamin A; 20 000 IU corresponds to 6 mg of vitamin A.

   Due to the different ways to express the levels of vitamin A, it was quite difficult to compare the results obtained in the available documentation and to adopt an uniform unit to express the concentrations of vitamin A.

3. Extensive animal studies have shown that vitamin A is required for normal growth and development. In food producing species, the minimum daily requirements range from 750 IU/kg feed (pregnant ewe) to 3 800 IU/kg feed (dairy cattle).

   At physiological concentrations vitamin A stabilises the cell membrane, stimulates the synthesis of certain proteins by acting on the transcription and possesses an electron transfer property.

   Vitamin A is essential for vision, growth, differentiation and proliferation of a very wide range of epithelial tissues, bone growth, reproduction and embryonic development.

4. Preformed vitamin A is found in animal products (liver, eggs and milk) and in fortified foods mainly as retinyl esters that are hydrolysed to retinol in the intestinal lumen after ingestion. Free retinol is then taken up by mucosal cells, bound to a specific cellular retinol-binding protein and esterified to long chain fatty acids to become mainly retinyl palmitate before being incorporated into chylomicrons for lymphatic transport to the liver. Provitamin A carotenoids (β-carotene), predominantly present in green and yellow vegetables and fruit, are directly taken up by enterocytes. After *in vivo* conversion, they are transported to the liver and other target tissues.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 84 47
E-mail: mail@emea.eu.int http://www.emea.eu.int

The absorption of retinol (rate 70 to 90%) is more efficient than that of carotenoids (rate 20 to 50%). With increasing amounts of dietary carotenoids ingested, the absorption can decrease to levels as low as 10%. Absorption efficiency of both retinol and carotenoids is dependent on a sufficient amount and quality of dietary lipids.

In the liver, where 50 to 80% of the body's total vitamin A is stored, retinol is taken up by hepatocytes and, if reserves are adequate, much of it is transferred to stellate cells for storage as retinyl esters. Normally, about 90 to 95% of the stored retinyl esters are found in stellate cells and 5 to 10% in hepatocytes from which retinol can be readily mobilised.

In humans, there is controversy about whether the minimal desirable amount of vitamin A liver stores is above 20 µg/g liver or above 30 µg/g liver (approximately 70 to 100 IU/g).

5. In a study carried out in rats, it was shown that the final mean liver concentrations of vitamin A (0.4 to 331 µg retinol/g; corresponding to 1.32 to 1 100 IU/g) depended both on the daily dose given (5 to 176 µg retinol) and on the length of the treatment period (7 to 12 weeks). Liver vitamin A concentrations were roughly proportional to the daily vitamin A intake. The serum concentrations of retinol increased with the amounts of retinol quantified in liver: 0.24, 0.40 and 0.44 to 0.71 µg/ml in serum versus 0.4, 40 and 331 µg/g in liver respectively. The mean ratio of the activity of retinol in serum to that in liver was 0.65 at daily retinol intake of 8 to 176 µg per day. The ratio did not vary according to the retinol liver reserves or vitamin A intake.

6. The oral LD_{50} varied according to the vitamin A derivatives. In mice, oral LD_{50} values ranged from 1 100 mg/kg bw to 26 000 mg/kg bw (3.7 million to 86 million IU/kg bw) for all-*trans*-retinoic acid and 13-*cis*-retinoic acid, respectively. In rats, the oral LD_{50} values were 2 000 mg/kg bw for all-*trans*-retinoic acid and 7 900 mg/kg bw for retinyl palmitate (6.6 million to 26 million IU/kg bw).

7. A few subchronic and chronic toxicity studies were performed in rats with vitamin A and its derivatives. All-*trans*-retinoic acid induced an increase in serum alkaline phosphatase after oral administrations of 0.4 mg/kg bw, for 90 days. After daily oral administrations of retinyl palmitate for 10 months at doses of 5.5 to 27.5 mg/kg bw, no adverse effects were reported. Retinol induced hyperglyceridaemia and extensive foci of degenerative myocardial fibers after daily oral administrations of 3 mg/kg bw for 3 months.

   No NOEL could be retained from the information provided.

8. Many published data reported the teratogenic potential of vitamin A and related compounds in almost all organ system and in several laboratory animals. In mice, oral administrations of 10 000 to 15 000 IU vitamin A given once between day 8 to 13 of gestation, induced cranial malformations. In monkeys, such malformations were observed after oral administrations of 10 to 45 mg/kg bw of all-*trans*-retinoic acid between days 21 and 45 of gestation. In hamster, malformations of the nervous system and cleft palate were reported after a single oral administration of 200 000 IU of vitamin A. In rats, oral doses of vitamin A (10 000 IU on day 9 and 10 of gestation or 160 000 IU from day 15 to 19 of gestation) induced malformations of the nervous system and cleft palates. All the vitamin A derivatives (all-*trans*-retinoic acid and 13-*cis*-retinoic acid) resulted in increases of foetal resorption, stillbirths and malformations of the foetuses. The type and the incidence of malformations observed depended on the species and on the stage of gestation (the effects were stronger during early pregnancy).

   No NOEL for teratogenicity could be established.

9. In humans, hypervitaminosis is characterised by fatigue, irritability, anorexia and loss of weight, vomiting and other gastro-intestinal disturbances, skin changes (alopecia, acne), anaemia, headache, pains in bone and joints. The clinical expression of symptoms of hypervitaminosis A requires very high dosages (2 to 5 million IU per adult per day). However, the hypervitaminosis A is rare in humans, and constituted scarce incidents. Moreover, the symptoms are quickly reversible after the withdrawal of administration of vitamin A.

Teratogenic effects of vitamin A was encountered in pregnant women who suffered of hypervitaminosis A. Abortions and malformations in foetuses were observed. A very low teratogenic risk appears possible from a chronic intake of 20 000 IU vitamin A per day and seems likely at doses exceeding 50 000 IU/day. Randomised, controlled intervention studies using multivitamin preparations containing up to 2.4 mg retinol (approximately 8 000 IU/day) have not shown any teratogenic potential of vitamin A and have, on the contrary, indicated a reduction of congenital malformations.

10. Dietary intake data obtained from nutrition surveys are of limited use in the case of vitamin A and provitamin A carotenoids because of the many factors that determine uptake from food such as the amount of circulating retinol, importance of liver stores, fat composition of the diet, amount of carotenoids or retinol present in food and food preparation. No international agreement has been achieved on the daily nutritional requirements for vitamin A "to sustain health in practically all healthy persons in a population". In industrialised countries the recommended intakes of vitamin A including provitamin A carotenoids can usually be obtained from the diet without problems.

    A study carried out in a US population excluding infants showed that total mean and the median daily intakes of vitamin A were close to 1.0 mg retinol (approximately 3 300 IU) and 0.624 mg retinol (2 100 IU), respectively. Of this amount, carotenoids and preformed vitamin A in terms of retinol contributed approximately to 25% and 75% of the total intake, respectively.

    The recommended dietary allowances of WHO, of Nutrition Board of the National Research Council, and of different European countries are in the same magnitude and depend on age and sex: 0.4 to 0.7 mg retinol/g (2 100 to 3 300 IU) for children, 0.5 to 1.0 mg retinol/g (2 500 to 5 000 IU) for men and 0.5 to 1.3 mg retinol/g (2 500 to 6 500 IU) for women.

11. The recommended safe upper limit of dietary intake for women, as suggested by the American Teratology Society is 3.0 mg retinol, corresponding to 10 000 IU. This value was also retained by the European Commission and WHO, who recommend this value as the upper limit of vitamin A during pregnancy. The CVMP retained this value of 3.0 mg Retinol Equivalents (corresponding to 10 000 IU) as the acceptable daily dose.

12. Published data on the depletion studies of the vitamin A were available. Significant amounts of vitamin A can be found in liver or muscle after treatment.

13. After a single intravenous injection to lambs of 4 800 µg of vitamin A acetate labelled with 384 µCi of tritium (approximately 15 000 IU per animal, corresponding to 470 IU/kg bw), no radioactivity could be found in urine and only traces were found in faeces. In lamb liver, levels of vitamin A of 0.260, 0.203 and 0.124 µg/kg were measured at 5, 33 and 61 days after intrajugular administration of 4.8 mg of vitamin A acetate. The half-life of vitamin A in liver was estimated to 75 days.

14. In cattle, after administration of food supplemented with vitamin A (0, 25, 50 and 100 IU/kg bw), it was shown that the vitamin A concentrations in liver of non-supplemented groups amounted to 0.040 and 0.066 µmol/g and increased up to 0.15 µmol/g after vitamin supply.

15. In pigs, after intramuscular administrations of 500 000 IU of vitamin A per animal in oily formulations, it was shown that 228 000 IU and 87 000 IU remained at the injection site after 25 and 32 days, respectively (no concentration per gram of muscle was given). At the same dosage (500 000 IU per animal), the vitamin A concentrations in liver were in the magnitude of 116 to 154 IU/g for oily formulation whereas they were higher, 353 to 500 IU/g with aqueous formulation.

    In another study, pigs received, by oral route, doses ranging from 50 000 IU to 500 000 IU/animal. The percentage of the administered dose present in the liver was higher when larger doses were given and was 70% of an individual dose of 500 000 IU, 54 to 65% of individual doses of 100 000 to 250 000 IU and 24% or less for an individual dose of 50 000 IU. At the highest dosage (500 000 IU/animal), vitamin A concentrations in liver were in magnitude of 630, 400 and 200 IU/g, at 4, 20 and 62 days after the administration, respectively.

16. In a Finnish survey, 186 samples of bovine and pork liver were analysed from July 1989 to May 1991 by HPLC in order to quantify vitamin A. The main vitamin A-active compounds found in both bovine and pork livers was retinyl palmitate (64% of the total vitamin A in bovine liver and 82% in pork liver). The average vitamin A levels expressed as retinol equivalents were found to be 288 µg/g for bovine liver (corresponding to 950 IU/g liver) and 255 µg/g for pork liver (corresponding to 850 IU/g liver). Of the bovine liver samples 80% had concentrations lower than 400 µg/g (corresponding to 1 350 IU/g liver); about 70 % of the pork liver had concentrations lower than 300 µg/g (corresponding to 1 000 IU/g liver).

    The results from a UK Ministry of Agriculture, Fisheries and Food (MAFF) survey of 649 liver samples revealed that the mean vitamin A concentrations calculated as retinol equivalents was 139 ± 96 µg/g (corresponding to 465 ± 320 IU/g) with a range of 3 to 1 267 µg/g liver. Mean values for individual species were: 188 µg/g (corresponding to 625 IU/g) in calf, 142 µg/g (corresponding to 475 IU/g) in ox, 173 µg/g (corresponding to 577 IU/g) in lamb, 174 µg (corresponding to 580 IU/g) in pig and: 97 µg/g liver (corresponding to 325 IU/g) in chicken.

    In France, a survey of 120 liver samples carried out in 1996 revealed that 70% of the liver samples contained more than 500 IU/g of vitamin A, the mean level being close to 715 IU/g

17. In the report of the Scientific Committee for Food on the Risk of Hypervitaminosis A (1991) the analytical data of the vitamin A content of the livers of animals raised for human consumption collected in the different Member States, showed a rather frequent occurrence of levels exceeding 1 000 IU/g liver, which might in certain cases reach even 4 000 IU/g liver. The opinion of the Scientific Committee for Food was that such levels constituted a risk for the health of the public and that immediate measures should be taken regarding animal husbandry practices in order to reduce rapidly and effectively the vitamin A content of the livers sold for consumption.

18. Recognising the potential of vitamin A for accumulation in liver, several EU Member States issued the health advice that pregnant women should not eat liver during the first two months of pregnancy.

Conclusions and recommendation

Having considered the criteria laid down by the Committee for the inclusion of substances in Annex II of Council Regulation (EEC) No 2377/90 and in particular that:

- vitamin A is an endogenously available substance,
- vitamin A is a normal component in the diet of humans and animals,
- vitamin A is used in veterinary medicine only for short-term therapy in individual animals only for the treatment of vitamin A deficiency,
- the animals are unlikely to be sent for slaughter during or immediately after treatment,
- the variable levels of vitamin A naturally present in edible tissues of animals would undoubtedly make the establishment of MRLs and their surveillance impracticable;

the Committee concludes that there is no need to establish an MRL for vitamin A and recommends its inclusion in Annex II to Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Vitamin A	All food producing species	

Considering the potential of vitamin A for accumulation in liver and injection site, a withdrawal period of appropriate length should be set.